

**Original araştırma (Original article)****Screening for resistance to *Heterodera filipjevi* (Madzhidov) Stelter (Tylenchida: Heteroderidae) and *Pratylenchus thornei* (Sher & Allen) (Tylenchida: Pratylenchidae) sister lines of spring wheat**

Bazı ekmeklik buğday hatlarının *Heterodera filipjevi* (Madzhidov) Stelter (Tylenchida: Heteroderidae) ve *Pratylenchus thornei* (Sher & Allen) (Tylenchida: Pratylenchidae)'ye karşı reaksiyonlarının araştırılması

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Breeding for resistance to the cereal cyst nematodes (CCN) *Heterodera filipjevi* (Madzhidov,) Stelter, and *H. avenae* (Wollenweber) and to the root lesion nematode (RLN) *Pratylenchus thornei* (Sher & Allen) is presently being undertaken by breeding programs at research institutions in Turkey. This study was carried out to screen for nematode resistance in an advanced spring bread wheat breeding population, 42 lines (F<sub>9</sub>) developed at CIMMYT in Mexico, by crossing resistant parent the Middle- Eastern landrace AUS4930 7.2 and susceptible parent, the widely adapted, high yielding CIMMYT line, Pastor. The results demonstrate that 31 lines are resistant to *P. thornei* and 5 lines are resistant to *H. filipjevi*. Only 4 of these lines (2, 7, 23 and 41) are resistant to both nematodes. Lines 2, 7 and 41 also contain the known resistance gene, *Cre1*. Although some lines carry the *Cre1* gene, they are susceptible to either both or one of these nematodes. There is no association among *H. filipjevi*, *P. thornei* and *Cre1* resistance due to differences in the resistance region in the plant genome.

**Key words:** Cereal cyst nematode, Root lesion nematode, AUS4930, *Cre1* gene

**Özet**

Türkiye'de buğday ıslah programlarında Tahıl kist nematodları, *Heterodera filipjevi* (Madzhidov) Stelter, *H. avenae* (Wollenweber) ve Kök lezyon nematodlarına (*Pratylenchus thornei* Sher & Allen) karşı dayanıklı çeşitlerin geliştirilmesi enstitülerce eşzamanlı olarak yürütülmektedir. Bu çalışmada CIMMYT-Mexico tarafından kullanılan dayanıklılık kaynağı AUS4930 7.2 ve yüksek verimli Pastor ebeveylelerinin melezlenmesinden elde edilen 42 (F<sub>9</sub>) adet melez hattın *P. thornei* and *H. filipjevi* karşı reaksiyonlarının belirlenmesi amaçlanmıştır. Denemeye alınan materyallerden 32 hat *P. thornei*'ye karşı, 5 hat ise *H. filipjevi*'ye karşı dayanıklı bulunmuştur. Her iki nematoda karşı dayanıklı bulunan 4 hattın (2, 7, 23 and 41) sadece 3 tanesinin (2, 7, 41) *Cre 1* genini taşıdığı bilinmektedir. Bazı hatlar *Cre1* genini taşımasına rağmen her iki nematoda veya nematodlardan bir tanesine karşı duyarlı bulunmuştur. Elde edilen sonuçlara göre *H. filipjevi* ve *P. thornei* ile *Cre1* geni dayanıklılıkları arasında buğday genomunda bulunan farklı dayanıklılık bölgelerinden dolayı bir ilişki bulunamamıştır.

**Anahtar sözcükler:** Tahıl kist nematodu, Kök yara nematodu, AUS4930, *Cre1* geni

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Alınış (Received): 11.01.2012

Kabul ediliş (Accepted): 30.03.2012

## Introduction

Plant parasitic nematodes cause major yield losses in wheat growing regions of the world. Cereal cyst nematodes (CCN) are sedentary and form cysts on the roots of cereal crops, while root lesion nematodes (RLN) migrate through roots, causing lesions and helping other root pathogens penetrate the root system (McDonald & Nicol, 2005). Nematode damage associated with both CCN and RLN are economically important in wheat production systems in several parts of the world, especially under rainfed or water stressed conditions (Williamson & Gleason, 2003; Nicol & Rivoal, 2008). Recent studies in Turkey have shown that CCN and RLN cause yield losses up to 40% and 70%, respectively (Toktay, 2008; Elekcioglu, 2009; Rivoal & Nicol 2009).

Many studies around the world have shown that nematode populations in cereals can be effectively reduced by using an IPM approach, and cultural practices, chemical control and biological control are already used to reduce the damage caused by plant parasitic nematode on cereals (Mitchinson et al., 2009; Rivoal & Nicol 2009; Singh et al., 2009). However, one of the most promising control methods is the identification and production of resistant germplasm, which can reduce nematode populations below economic thresholds (Toktay et al., 2006; Nicol et al., 2009; Dababat et al., 2011). Resistance to nematodes in plants is defined as the capacity of the host plant to prevent or reduce the multiplication of the nematode (Rathjen et al., 1998). The Iraqi landrace AUS4930 is resistant to both the CCN *H. avenae* (Australian pathotype Ha13) and the Turkish *H. filipjevi* (pathotype HF1) and to the root lesion nematode *Pratylenchus thornei* (Nicol & Rivoal, 2000; Toktay, 2008; Rivoal & Nicol, 2009; Sahin, 2010). Pastor is a high yielding and widely adapted spring wheat variety developed by CIMMYT and grown in Turkey. It is however susceptible to both CCN and RLN. These two parents were used to create a population in which we hoped to find resistance to both *H. filipjevi* and *P. thornei*. A total of 42 advanced spring bread wheat (F<sub>9</sub>) breeding lines was developed by CIMMYT in Mexico using Pastor and AUS4930 7.2. Some of the crosses of AUS4930 7.2 contain the *Cre1* gene, which confers resistance to most European *H. avenae* pathotypes and to the Australian *H. avenae* pathotype Ha13 (De Majnik et al., 2003).

The objective of this study was to screen the 42 sister lines derived from crosses between AUS4930 7.2 and Pastor for resistance to Turkish pathotypes of both *H. filipjevi* and *P. thornei*.

## Material and Methods

A population of advanced spring wheat lines derived from AUS4930 7.2 x Pastor was produced and homogenized to F<sub>9</sub> level at CIMMYT in Mexico. A total of 42 sister lines were obtained from this breeding program and screened for the resistance gene *Cre1*, using molecular markers (Toktay, 2008). These lines were then transferred to Turkey where an improved *in vitro* screening method as described by Toktay (2008) and Kiel et al. (2009) was used to test for resistance to Turkish *P. thornei* and *H. filipjevi* isolates. The F<sub>9</sub> breeding lines screened are listed in Table 1.

The improved *in vitro* screening method for resistance to CCN and LRN in cereals involves growing cereals from seed in tubes, infecting the roots with nematodes and evaluating the population of nematodes after a given time. In our screening test, screening tubes (100 mm long x 15 mm in diam. for RLN; 150 mm long x 30 mm in diam. for CCN) were filled with a mixture of sterilized sand, field soil and organic matter (70:29:1 v/v). One sterilized and pre-germinated seed with approximately 3 equidistant, 1-cm long seminal roots was planted per screening tube.

The *P. thornei* population came from a single nematode collected in a wheat field in Adana, Turkey that was cultured on carrot discs. One week after planting, each *P. thornei* screening tube was inoculated with four hundred *P. thornei* (J2, J3, J4 and adults) in 1 ml of water. *H. filipjevi* (HF1) cysts were collected from an infected wheat field in the Central Anatolian Plateau. A total of 200 freshly hatched J2 were inoculated per *H. filipjevi* screening tube: 100 J2 at planting time and another 100 J2 24 hours after the first inoculation.

Table 1. List of F<sub>9</sub> breeding lines derived from AUS4930 7.2 x Pastor crosses and screened in this study

No	Breeding lines name	CID*	SID**	Cre1*** (Toktay, 2008)
1	AUS4930 7.2 x PASTOR	431784	202	+
2	AUS4930 7.2 x PASTOR	431784	204	+
3	AUS4930 7.2 x PASTOR	431784	205	+
4	AUS4930 7.2 x PASTOR	431784	208	-
5	AUS4930 7.2 x PASTOR	431784	213	-
6	AUS4930 7.2 x PASTOR	431784	216	-
7	AUS4930 7.2 x PASTOR	431784	217	+
8	AUS4930 7.2 x PASTOR	431784	261	-
9	AUS4930 7.2 x PASTOR	431784	282	+
10	AUS4930 7.2 x PASTOR	431784	283	-
11	AUS4930 7.2 x PASTOR	431784	284	-
12	AUS4930 7.2 x PASTOR	431784	290	-
13	AUS4930 7.2 x PASTOR	431784	293	-
14	AUS4930 7.2 x PASTOR	431784	305	-
15	AUS4930 7.2 x PASTOR	431784	306	-
16	AUS4930 7.2 x PASTOR	431784	309	-
17	AUS4930 7.2 x PASTOR	431784	313	-
18	AUS4930 7.2 x PASTOR	431784	314	-
19	AUS4930 7.2 x PASTOR	431784	317	-
20	AUS4930 7.2 x PASTOR	431784	319	-
21	AUS4930 7.2 x PASTOR	431784	322	-
22	AUS4930 7.2 x PASTOR	431784	323	-
23	AUS4930 7.2 x PASTOR	431784	324	-
24	AUS4930 7.2 x PASTOR	431784	325	-
25	AUS4930 7.2 x PASTOR	431784	329	+
26	AUS4930 7.2 x PASTOR	431784	331	-
27	AUS4930 7.2 x PASTOR	431784	332	-
28	AUS4930 7.2 x PASTOR	431784	333	-
29	AUS4930 7.2 x PASTOR	431784	338	-
30	AUS4930 7.2 x PASTOR	431784	341	+
31	AUS4930 7.2 x PASTOR	431784	349	+
32	AUS4930 7.2 x PASTOR	431784	350	+
33	AUS4930 7.2 x PASTOR	431784	353	+
34	AUS4930 7.2 x PASTOR	431784	357	-
35	AUS4930 7.2 x PASTOR	431784	375	+
36	AUS4930 7.2 x PASTOR	431784	378	-
37	AUS4930 7.2 x PASTOR	431784	379	-
38	AUS4930 7.2 x PASTOR	431784	385	+
39	AUS4930 7.2 x PASTOR	431784	395	+
40	AUS4930 7.2 x PASTOR	431784	396	+
41	AUS4930 7.2 x PASTOR	431784	399	+
42	AUS4930 7.2 x PASTOR	431784	405	-

\* Cross identification number. \*\* Selection identification number. \*\*\* +: *Cre1* gene present; -: *Cre1* gene not present.

Seven replicates of each F<sub>9</sub> line and of each parent (AUS4930 7.2 and Pastor) were tested in a randomized complete block design. Plants were grown in a controlled conditions room with 16 hours of supplementary artificial light, temperatures between 20 - 25°C and 70% relative humidity in 2010. Plants were bottom watered as needed to maintain soil moisture and were harvested 9 weeks after nematode inoculation.

At harvest, shoots were removed and *P. thornei* vermiform nematodes were extracted from roots and soil using the modified Baermann funnel and mister extraction method (Southey, 1986), while the Fenwick can method (Fenwick, 1940) was used to extract *H. filipjevi* cysts from soil and roots. The total *P. thornei* numbers and *H. filipjevi* cysts on both root and soil was counted under microscope for each plant after extraction.

Data was analysed with analysis of variance using SPSS 17.0 for Windows (SPSS Inc., Illinois, USA). Differences among treatments were tested using one-way analysis of variance (ANOVA) followed by the Tukey Test for comparison of means, if the F-value was significant at  $P < 0.05$ .

## Results and Discussion

A population generated by crossing the resistant AUS4930 7.2 line and the susceptible but high yielding Pastor line was screened for resistance to Turkish isolates of RLN (*P. thornei*) and CCN (*H. filipjevi*) under controlled conditions. Molecular resistance screening carried out in Mexico prior to the *in vitro* resistance screening study in Turkey revealed that 15 of the 42 lines in the F<sub>9</sub> population contain the CCN resistance gene *Cre1* (Toktay, 2008).

Thirty one lines of the F<sub>9</sub> population were found to be resistant to *P. thornei*; six lines were moderately resistant while five lines were susceptible (Figure 1). The most resistant line was the parent AUS4930 7.2 but the other parent Pastor was highly susceptible. The highest number of *P. thornei* per plant (1179.79 vermiform *P. thornei*/plant) was recovered from line 29 (Table 2).

Five lines of the F<sub>9</sub> population were determined to be resistant and eight lines moderately resistant, while twenty nine were found to be susceptible to the Turkish *H. filipjevi* isolate used. AUS 4930 7.2 and Pastor had 3 and 8 cysts/plant, respectively. The most *H. filipjevi*-resistant line was 30, with an average of 0.43 cysts/plant, while the least resistant line was 15, with 9 cysts/plant (Figure 2).

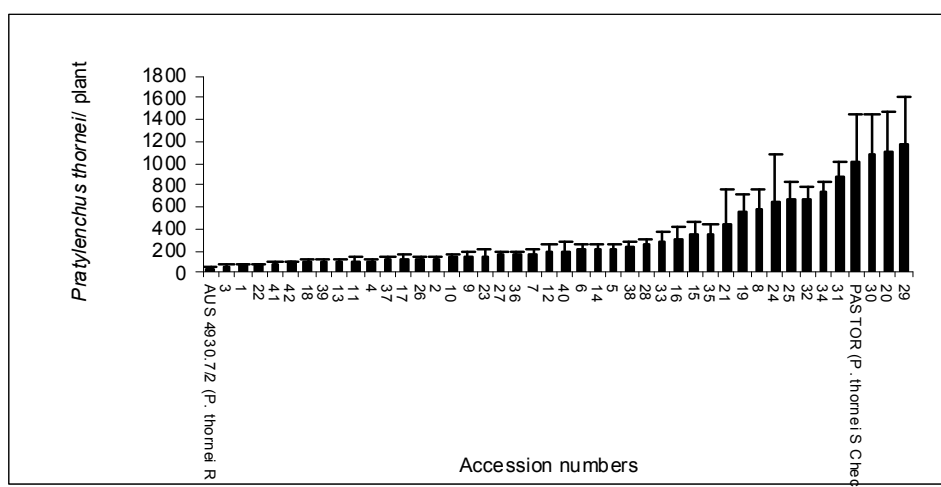


Figure 1. Total number of *Pratylenchus thornei* per plant in 42 AUS4930 7.2 x Pastor sister lines and in resistant and susceptible parents (AUS4930 7.2 and Pastor, respectively).

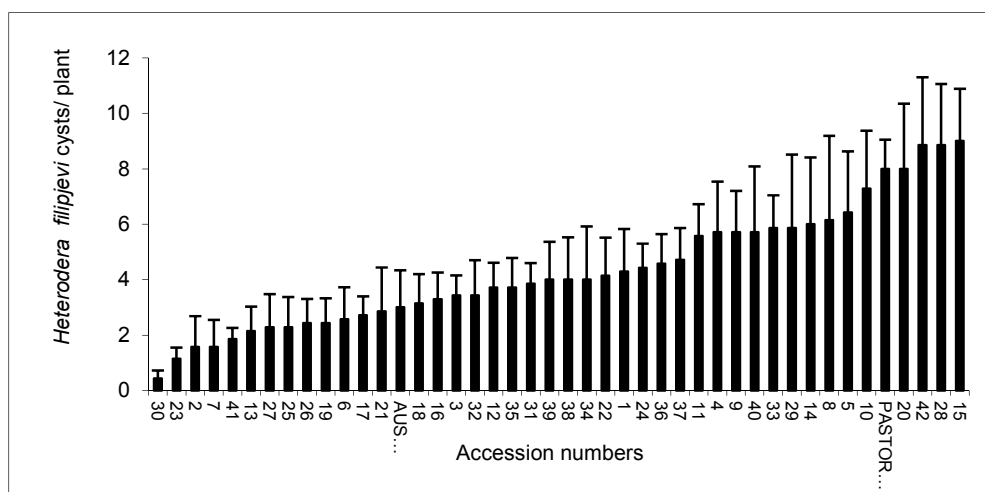


Figure 2. Total number of *Heterodera filipjevi* cysts per plant in 42 AUS4930 7.2/Pastor sister lines and in resistant and susceptible parents (AUS4930 7.2 and Pastor, respectively).

Our testing revealed that 11 of the 15 F<sub>9</sub> sister lines containing the *Cre1* gene are resistant to *P. thornei* and four of them are resistant to *H. filipjevi*. Three of the *Cre1*-containing lines (2, 7 and 41) are resistant to both *P. thornei* and to *H. filipjevi* (Table 2). The only other line resistant to both nematodes was line 23 but it does not contain the resistance gene *Cre1*.

Table 2. Total number of vermiform *Pratylenchus thornei* and *Heterodera filipjevi* cysts per plant in 42 AUS4930 7.2/Pastor sister lines and in resistant and susceptible parents (AUS4930 7.2 and Pastor, respectively), 9 weeks after nematode inoculation

No	SID	<i>Cre1</i> <sup>3</sup>	<i>Pratylenchus thornei</i>		<i>Heterodera filipjevi</i>	
			Mean $\pm$ SE <sup>1</sup>	Reaction <sup>2</sup>	Mean $\pm$ SE	Reaction
1	202	+	70.93 $\pm$ 18.56 a <sup>4</sup>	R	4.29 $\pm$ 1.54 c	S
2	204	+	134.25 $\pm$ 22.66 a	R	1.57 $\pm$ 1.11 a	R
3	205	+	57.43 $\pm$ 15.22 a	R	3.43 $\pm$ 0.72 c	S
4	208	-	110.50 $\pm$ 22.83 a	R	5.71 $\pm$ 1.82 d	S
5	213	-	220.39 $\pm$ 38.43 a	R	6.43 $\pm$ 2.19 d	S
6	216	-	204.86 $\pm$ 57.66 a	R	2.57 $\pm$ 1.15 b	MR
7	217	+	175.25 $\pm$ 49.73 a	R	1.57 $\pm$ 0.97 a	R
8	261	-	569.89 $\pm$ 187.46 a	MR	6.14 $\pm$ 3.04 d	S
9	282	+	146.46 $\pm$ 48.64 a	R	5.71 $\pm$ 1.49 d	S
10	283	-	136.79 $\pm$ 32.91 a	R	7.29 $\pm$ 2.09 d	S
11	284	-	108.29 $\pm$ 29.69 a	R	5.57 $\pm$ 1.15 d	S
12	290	-	186.67 $\pm$ 74.78 a	R	3.71 $\pm$ 0.89 c	S
13	293	-	107.54 $\pm$ 14.73 a	R	2.14 $\pm$ 0.88 b	MR
14	305	-	209.54 $\pm$ 59.70 a	R	6.00 $\pm$ 2.41	S
15	306	-	340.32 $\pm$ 125.30 a	R	9.00 $\pm$ 1.89 e	S
16	309	-	298.29 $\pm$ 127.79 a	R	3.29 $\pm$ 0.97 c	S
17	313	-	120.71 $\pm$ 46.29 a	R	2.71 $\pm$ 0.68 b	MR
18	314	-	90.64 $\pm$ 27.98 a	R	3.14 $\pm$ 1.06 c	S
19	317	-	553.86 $\pm$ 171.98 b	MR	2.43 $\pm$ 0.90 b	MR
20	319	-	1107.00 $\pm$ 362.08 e	S	8.00 $\pm$ 2.35 e	S
21	322	-	451.21 $\pm$ 302.40 e	MR	2.86 $\pm$ 1.58 b	MR
22	323	-	71.39 $\pm$ 14.94 a	R	4.14 $\pm$ 1.37 c	S
23	324	-	153.57 $\pm$ 63.02 a	R	1.14 $\pm$ 0.40 a	R
24	325	-	659.25 $\pm$ 412.54 b	MR	4.43 $\pm$ 0.87 c	S
25	329	+	663.68 $\pm$ 158.40 b	MR	2.29 $\pm$ 1.08 b	MR
26	331	-	126.86 $\pm$ 20.49 a	R	2.43 $\pm$ 0.87 b	MR
27	332	-	159.71 $\pm$ 33.56 a	R	2.29 $\pm$ 1.19 b	MR
28	333	-	267.57 $\pm$ 35.73 a	R	8.86 $\pm$ 2.20 e	S
29	338	-	1179.79 $\pm$ 421.05 e	S	5.86 $\pm$ 2.65 d	S
30	341	+	1089.46 $\pm$ 353.07 e	S	0.43 $\pm$ 0.30 a	R
31	349	+	870.39 $\pm$ 148.66 d	S	3.86 $\pm$ 0.74 c	S
32	350	+	668.82 $\pm$ 112.60 b	MR	3.43 $\pm$ 1.27 c	S
33	353	+	288.96 $\pm$ 84.10 a	R	5.86 $\pm$ 1.18 d	S
34	357	-	744.25 $\pm$ 77.55 c	S	4.00 $\pm$ 1.91 c	S
35	375	+	361.43 $\pm$ 78.08 a	R	3.71 $\pm$ 1.06 c	S
36	378	-	166.29 $\pm$ 35.30 a	R	4.57 $\pm$ 1.07 c	S
37	379	-	120.39 $\pm$ 25.14 a	R	4.71 $\pm$ 1.15 c	S
38	385	+	237.39 $\pm$ 50.17 a	R	4.00 $\pm$ 1.53 c	S
39	395	+	100.79 $\pm$ 28.14 a	R	4.00 $\pm$ 1.36 c	S
40	396	+	196.57 $\pm$ 86.04 a	R	5.71 $\pm$ 2.38 d	S
41	399	+	88.93 $\pm$ 22.93 a	R	1.86 $\pm$ 0.40 a	R
42	405	-	89.88 $\pm$ 14.01 a	R	8.86 $\pm$ 2.44 e	S
43	AUS4930 7.2	+	36.93 $\pm$ 9.98 a	R	3.00 $\pm$ 1.34 a	MR
44	PASTOR	-	1021.86 $\pm$ 418.60 e	S	8.00 $\pm$ 1.05 e	S

<sup>1</sup> SED: Standard Error Degree

<sup>2</sup> R: Resistant; MR: Moderately Resistant; S: Susceptible.

<sup>3</sup> +: *Cre1* gene present; -: *Cre1* gene not present.

<sup>4</sup> Means with the same letter, in the same column, are not significantly different at P = 0.05, using the Tukey test.

The results demonstrate that 74% (31 lines) of the forty-two F<sub>9</sub> lines derived from crosses between AUS4930 7.2 and Pastor (42 lines) are resistant to *P. thornei* and 12% of the population (5 lines) are resistant to *H. filipjevi*. Some lines are only moderately resistant to *P. thornei* (6 lines or 14%) and to *H. filipjevi* (8 lines or 19%).

Two lines and ten lines of the population were susceptible to *P. thornei* and of and *H. filipjevi*, respectively, even though they contain the resistance gene *Cre1*. Line 31 contains the resistance gene *Cre1*, but is susceptible to both nematodes. Line 27 does not contain *Cre1* but is nonetheless resistant to *P. thornei* and moderately resistant to *H. filipjevi*, while line 23 is the only line that does not contain *Cre1* but is nonetheless resistant to both tested nematodes.

This study shows that there is no clear relationship between resistance to the Turkish *P. thornei* and *H. filipjevi* isolates and the CCN-resistance gene *Cre1*. The cereal cyst nematode resistance gene *Cre1* is effective against the Australian *H. avenae* pathotype Ha 13 and is actively being utilized in marker-assisted selection breeding programs and has been released in commercial cultivars (Vanstone et al., 2008; Akar et al., 2009; Rivoal & Nicol 2009). Commercial Turkish cultivars and breeding lines have been screened for the presence of the *Cre1* gene but it was not found (Akar et al., 2009; Özarslandan et al., 2010). There is no commercial cultivar resistant to *H. filipjevi* or *P. thornei* in Turkey. This gene is not effective against the tested Turkish populations of *H. filipjevi* and *P. thornei*. This study therefore indicates that the Turkish populations of RLN and CCN can overcome the resistance conferred to wheat plants by the *Cre1* gene, as also reported by Özarslandan et al. (2010) and Şahin (2010).

Recently, resistance to CCN has been well documented to be controlled by a single gene, whilst RLN resistance is quantitative and controlled by a number of genes (Toktay et al., 2006; Nicol et. al., 2009). The present study indicates that the resistant genes in AUS4930 7.2 is allelic or closely related to the published *Cre1* on chromosome 2B (Toktay et al., 2006). Although resistant regions in AUS4930 7.2 against Cereal cyst nematode and Root lesion nematode may share one common chromosomal region, there is no suggestion this relates to the genetic control of both nematodes. More detailed work is required on this new resistance line to determine if the resistance region is linked to different resistance loci. Nevertheless, results from the present study have useful implications for wheat breeding programs as they suggest that higher levels of resistance are possible when resistance loci on different wheat chromosomes are combined.

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